Pre-clinical Combination of AO-176, a Highly Differentiated Clinical Stage CD47 Antibody, with Either Azacitidine or Venetoclax Significantly Enhances DAMP Induction and Phagocytosis of Acute Myeloid Leukemia

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Disclosures

• All authors are employees of Arch Oncology, Inc.
### AO-176: Clearly Differentiated in the CD47 Landscape

**Humanized IgG2 anti-CD47 Antibody with Multiple Mechanisms of Action**

<table>
<thead>
<tr>
<th>CONVENTIONAL ANTI-CD47 APPROACH</th>
<th>AO-176</th>
<th>POTENTIAL ADVANTAGES</th>
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</thead>
<tbody>
<tr>
<td><strong>1 Blocking</strong></td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>2 Preferential Binding to Tumor Cells vs. Normal Cells</strong></td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td><strong>3 Better Binding in Tumor Environment (Low pH)</strong></td>
<td>X</td>
<td>✓</td>
</tr>
<tr>
<td><strong>4 Direct Killing &amp; DAMP Induction</strong></td>
<td>X</td>
<td>✓</td>
</tr>
</tbody>
</table>

**Differentiated Best-in-Class Antibody | Blocking and Direct Killing | Unique Among the Anti-CD47 Field**

Azacitidine Enhances AO-176-Mediated Phagocytosis of HL-60 Cells

AO-176 Increases Phagocytosis When Combined with Azacitidine. Human monocyte derived macrophages were plated at a concentration of 5 x 10⁴ cells per well in a 96 well plate. 8 x 10⁴ CFSE (1μM) labeled human HL-60 acute myeloid leukemia cells were treated with 3 or 10μM azacitidine overnight prior to being incubated with increasing concentrations of AO-176 and added to the macrophage cultures at 37°C for two hours. Non-phagocytosed target tumor cells were removed, and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14 prior to analysis by flow cytometry. Percent (%) phagocytosis is calculated from the ratio of CFSE+/CD14+ to total CD14+ macrophages. Figures show single concentrations of each agent alone, or in combination, as optimized per each cell line. Azacitidine also increased surface exposure of calreticulin. Two other AML cell lines, MV4-11 and KG-1 showed similar effects.
Venetoclax Enhances AO-176-Mediated Phagocytosis of HL-60 Cells

AO-176 Increases Phagocytosis When Combined with Venetoclax. Human monocyte derived macrophages were plated at a concentration of 5 x 10⁴ cells per well in a 96 well plate. 8 x 10⁴ CFSE (1μM) labeled human HL-60 acute myeloid leukemia cells were treated with 3 or 10nM venetoclax overnight prior to being incubated with increasing concentrations of AO-176 and added to the macrophage cultures at 37°C for two hours. Non-phagocytosed target tumor cells were removed, and macrophage cultures were washed extensively. Macrophages were trypsinized and stained for CD14 prior to analysis by flow cytometry. Percent (％) phagocytosis is calculated from the ratio of CFSE+/CD14+ to total CD14+ macrophages. Figures show single concentrations of each agent alone, or in combination, as optimized per each cell line. Venetoclax also increased surface exposure of calreticulin. Two other AML cell lines, MV4-11 and KG-1 showed similar effects.
AO-176 Combines with Azacitidine to Increase AML Cell Killing

AO-176 Enhances Cell Killing in Combination with Azacitidine. HL-60 (left panel) or MV4-11 (right panel) acute myeloid leukemia cells were incubated with 100 µg/mL AO-176 alone, 5 µM azacitidine alone, or a combination of AO-176 and azacitidine in RPMI media at 37°C for 24 hours. Cells were washed and then stained with Annexin V PE and SYTOX Blue followed by flow cytometry analysis.
AO-176 Combines with Venetoclax to Increase AML Cell Killing

AO-176 Enhances Cell Killing in Combination with Venetoclax. MV4-11 or KG-1 acute myeloid leukemia cells were incubated with 100 µg/mL (MV4-11, left panel) or 10 µg/mL (KG-1, right panel) AO-176 alone, 0.3 µM (MV4-11) or 2.5 µM (KG-1) venetoclax alone and their respective combinations with AO-176 in RPMI media at 37°C for 24 hours. Cells were washed and then stained with Annexin V PE and SYTOX Blue followed by flow cytometry analysis.
AO-176 Increases HL-60 Surface DAMP Exposure, Alone and In Combination with Azacitididine

AO-176 Enhances DAMP Induction. Left panel-HL-60 acute myeloid leukemia cells were incubated with 10, 30 or 100 µg/mL AO-176 alone in RPMI media at 37°C for 24 hours. Cells were washed and then stained for calreticulin and viability (SYTOX Blue) followed by flow cytometry analysis. Cell surface exposure of calreticulin was increased by treatment with AO-176 in a concentration-dependent manner.

Right Panel-AO-176 Enhances DAMP Induction in Combination with 5-Azacitididine. HL-60 acute myeloid leukemia cells were incubated with 100 µg/mL AO-176 alone, 5 µM azacitidine alone, or a combination of AO-176 and azacitidine in RPMI media at 37°C for 24 hours. Cells were washed and then stained for PDIA3 and viability (SYTOX Blue) followed by flow cytometry analysis. PDIA3 cell surface exposure was increased by AO-176 treatment and further enhanced in combination with azacitidine.
AO-176 Treatment of HL-60 Xenograft Mice Results in Significant Increases in Survival, Alone and In Combination with Azacitidine

Human HL-60 acute myeloid leukemia cells were implanted intravenously into NSG mice (N=10/group) and treatment was initiated four days after implantation. Human IgG2 (hIgG2), 2.5% DMSO:PBS solution, azacitidine, AO-176, 5F9, Aza and AO-176 or Aza and 5F9 in combination were administered by intraperitoneal (IP) injection and survival was monitored and plotted versus days following tumor inoculation. **** p<0.0001
AO-176 is Efficacious in Pre-Clinical AML Models

1. Azacitidine and venetoclax enhance AO-176-mediate phagocytosis of AML cells

2. AO-176 combines with azacitidine and venetoclax to increase AML cell killing

3. AO-176 induces cell surface DAMP exposure, alone and in combination, with azacitidine on HL-60 cells

   AO-176, alone and in combination with azacitidine, significantly increases survival in an HL-60 xenograft model. AO-176 activity is comparable to that of a magrolimab benchmark antibody.

4. Taken together, these data highlight the strong therapeutic potential of AO-176 for AML patients.

AO-176 is currently being evaluated in two phase 1/2 studies for the treatment of solid tumors (NCT03834948) and multiple myeloma (NCT044445701).